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A COMPARISON OF ARTIFICIAL AND NATURAL GASTRIC DIGESTION, TOGETHER WITH A STUDY OF THE DIFFUSIBILITY OF PROTEOSES AND PEPTONE.

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(*Contributions from the Sheffield Biological Laboratory of Yale University.*)

It is a well-understood fact that the normal digestive processes going on in the living alimentary tract take place under quite different conditions from those which exist ordinarily in artificial digestion experiments. At the same time, physiologists have been wont to assume that so far as the purely chemical part of the process is concerned, the results are essentially the same. Minor differences might naturally be expected, especially in the rate of action, for when, as in natural gastric digestion, proteolytic action takes place under such conditions that the products of digestion are continually being removed by absorption and fresh digestive juice continually secreted, it is obvious that a more rapid proteolytic action might be looked for than in the confined limits of a beaker or a flask where the products of digestion must necessarily accumulate, and where the digestive juice is limited to the original quantity.

Granting, therefore, that in general the chemical changes incidental to digestion may take place more rapidly in the natural than in the artificial process, the important question connected therewith is, how far will this possible difference in the rate of action affect the character or proportion of the normal end-products of digestion? In a previous article[1] on the relative formation of proteoses and peptone in gastric digestion, it was shewn by a series of quantitative experiments with various proteids that in their digestion with pepsin-hydrochloric acid, even under the most favorable circumstances, the formation of peptone

[1] Chittenden and Hartwell. This *Journal.* Vol. XII. p. 12.

is a gradual process, and that the greater part, if not all, of the peptone thus formed passes through the intermediate stages of primary and secondary proteose. It was further shewn that at the end of even the most vigorous and long-continued digestion, a considerable portion of the proteid digested still existed in the form of proteose. In other words, the results fully substantiated the view frequently advanced[1], that in the artificial gastric digestion of any ordinary proteid, complete peptonization rarely, if ever, occurs; the primary proteoses are first formed, these pass slowly by hydration into the secondary proteose and the latter in turn into true peptone, so that under no ordinary circumstances can an artificial gastric digestion be so conducted as to have the proteoses give place entirely to peptone. How far is this true of natural gastric digestion?

It is obviously impossible to so conduct an artificial digestion experiment as to have the conditions exactly the same as those of the natural process. In artificial digestions, as ordinarily conducted, the most important factor in bringing about a possible inhibition of ferment action is the accumulation of the products of digestion. Certainly, this constitutes one of the most important points of difference between the natural and the artificial process. This has been very ably discussed by Sheridan Lea in a recent paper[2], where results are detailed shewing the effects of the removal of the digestive products on the amylolytic action of saliva and on the proteolytic action of alkaline trypsin solutions. Thus, Lea finds that "when the digestion of starch by saliva is carried on under conditions which ensure a very considerable removal of the products (maltose) as they are formed, then the rate at which the digestion takes place is largely increased, the total amount of starch converted into sugar is much greater, and the residue of dextrin is much less than under conditions, otherwise similar, when the products are not removed." A more important conclusion, however, based on the results obtained by this study of the influence of the removal of the digestive products on the relative amounts of dextrin and maltose formed in salivary digestion, is that "by prolonged digestion of larger masses of starch or the shorter digestion of smaller quantities, the total amount of sugar which is formed and the small amount of dextrin which is

[1] Kühne and Chittenden. *Zeitschrift für Biologie*, Bd. xix. p. 159; Bd. xx. p. 11; Bd. xxii. p. 409. Chittenden. *Studies from Lab. Physiol. Chem.* Yale University, Vols. 2, 3.

[2] "A Comparative Study of Artificial and Natural Digestions." This *Journal*, Vol. xi. p. 226.

simultaneously produced justifies the assumption that under the more favorable conditions existing in the alimentary canal, starch is completely converted into sugar before absorption." If this is true of salivary digestion, may not a like assumption be applied to gastric digestion? May it not be that the lack of complete peptonization seen in artificial gastric digestion is due to accumulation of the products of digestion, and that in natural digestion where rapid and complete removal of the products is accomplished by means of the marvellous activity of the living epithelial cells, a far greater proportion of proteid-food may reach the peptone-stage? Obviously, we cannot by any means at our disposal imitate this selective activity of the living cells, and must depend entirely upon the physical property of diffusibility which the products shew. But inasmuch as the primary changes impressed on alimentary principles by the digestive ferments are not, chemically speaking, of a profound character, but affect much more the physical state of these principles than their chemical composition, investigation of their diffusibility and the influence of their removal from the digestive mixture on the activity of the ferment may prove of considerable interest and profit.

It has been, therefore, the main object of the present study to ascertain by experiment how far the action of pepsin-hydrochloric acid on proteids is influenced by the partial removal of the products of digestion as they are formed, and whether or no, under such conditions, complete peptonization is possible. Further, the results thus obtained have been verified to a certain extent by a study of the relative proportion of proteoses and peptone in the contents of the human stomach after a purely proteid diet.

1. *Comparison of the proteolytic action of pepsin-hydrochloric acid in a flask with that in a dialyzer.*

In the artificial digestion experiments in flasks previously recorded[1] it was found a difficult matter to obtain from a natural proteid much more than 50 per cent. of peptone, even by the use of strong pepsin solutions and an abundance of dilute acid. Indeed, beyond a certain point, the introduction of an additional amount of dilute acid, even with an increase of pepsin, was not accompanied by any increase in the formation of peptone, thus perhaps indicating that the lack of complete peptonization there noted was not due, wholly at least, to accumulation

[1] Chittenden and Hartwell. *Loc. cit.*

of the products of digestion. Here, however, by carrying on a series of digestions in parchment tubes suspended in warm dilute acid a more thorough test of this point has been made.

Methods employed. The apparatus employed was similar to that used by Lea[1] in his experiments with saliva, except that it was considerably larger, and hence capable of holding a much larger quantity of fluid. It consisted of a glass cylinder 19 inches high and 5 inches in diameter, with a capacity of about 5 litres, having a small tubulure near the bottom, into which was fitted by means of a rubber cork a long glass tube with a stopcock by which the contents of the vessel could be drawn off at will. During the experiment, this cylinder was filled with dilute hydrochloric acid of the same strength as that contained in the artificial gastric juice, while in the acid fluid was suspended a loop of parchment paper tube[2] within which were placed the pepsin-hydrochloric acid and the proteid to be digested.

This cylinder was fitted into a larger cylinder of glass 20 inches high and 8 inches in diameter, of 17 litres capacity, the space between the two cylinders being filled in with water which was kept at a constant temperature of 38° C. by a current of heated water, which in turn was kept fairly constant by being passed through a long coiled pipe or worm immersed in water of a higher temperature. In this manner, after a little practice, the temperature of the fluids in the inner cylinder; *i.e.*, the digestive mixture in the parchment tube and the surrounding fluid into which the products of digestion diffused, was kept quite constant at 38° C. Further, when desired a peristaltic-like movement could be communicated to the contents of the parchment tube by connecting it with a motor by means of a cord, as suggested by Lea.

The proteids employed in the several series of experiments were coagulated egg-albumin, liquid egg-albumin and purified blood-fibrin. The coagulated egg-albumin was prepared by taking the whites of the necessary number of eggs, rupturing the enclosing membranes, diluting somewhat with water, and then coagulating the filtered fluid by pouring it into a large volume of boiling water acidulated with a little acetic acid. The resulting coagulum was then washed thoroughly with boiling water, pressed as dry as possible, and when ready for use a sampled portion was dried at 110° C. until of constant weight, in order

[1] *Loc. cit.* p. 227.

[2] This dialyzer-tube was such as has long been used by Kühne, and was obtained from the manufacturer, C. Brandegger of Ellwangen.

to determine the content of dry albumin. Obviously, this residue contained in addition to the proteid matter some ash, but this was too small in quantity to need any consideration. Thus, one portion of the moist albumin coagulum weighing 10·5028 grams, equivalent to 1·2596 grams of dry albumin, yielded on ignition 0·0217 gram ash, or about 0·2 per cent. calculated on the original coagulum, or 1·7 per cent. of the dry proteid.

In the determination of the several products of digestion, the methods employed were much the same as those made use of on a former occasion[1]. When it was desired to stop a digestion, the ferment was killed by heating the solution to boiling. On cooling, the insoluble antialbumid was determined by collecting it on a weighed filter, washing it thoroughly with water, lastly with alcohol and ether and drying it at 110° C. until of constant weight. In the acid filtrate, the neutralization precipitate was determined by careful neutralization with pure sodium carbonate, the mixture heated to near boiling and the precipitate collected on a weighed filter, thoroughly washed and dried at 110° C.

The neutral filtrate and washings, containing the proteoses and peptone, were concentrated to a small volume, a drop or two of acetic acid added, and the fluid saturated while boiling hot with pure crystallized ammonium sulphate. The hot fluid could generally be decanted from the gummy precipitate of proteoses which adhered to the sides of the vessel. The albumoses were then redissolved in a small amount of water and again precipitated with ammonium sulphate, after which they were washed as thoroughly as possible with a hot saturated solution of the ammonium salt. In this manner, both peptone and sodium chloride[2], which naturally adhered to the more or less gummy precipitate, were completely removed. Whether this method of separation gives absolutely correct results need not be discussed here. This much, however, is certain, that it is the most accurate method at present readily available, and in our opinion such error as may exist consists rather in an incomplete separation of a portion of the deuteroproteose than in a partial precipitation of the peptone. The precipitate of proteoses, together with the adherent ammonium sulphate, was dissolved in a little hot water and the solution, with any undissolved particles of hetero or dysalbumose carefully rinsed into a small weighed capsule, in

[1] Chittenden and Hartwell. This *Journal*. Vol. XII. p. 15.

[2] The complete freedom of the proteose-precipitate from sodium chloride was always made sure of, since the accuracy of the method employed depends in great part upon the freedom of this precipitate from everything but proteoses and ammonium sulphate.

which it was evaporated to dryness on a water-bath and then dried in an air bath at 110° C. until of constant weight. To determine the ammonium sulphate contained in the dried proteoses, the residue, after the final weight, was dissolved in hot water, the solution filtered from any insoluble residue and then made up to some definite volume, usually 500 c.c. Of this solution, two portions, of 50 or 100 c.c. each, were drawn off with a pipette, diluted suitably with water, made faintly acid with dilute hydrochloric acid, and the sulphuric acid precipitated from the hot solution with barium chloride after the ordinary method. From the weight of barium sulphate thus obtained the equivalent in ammonium sulphate was calculated, and the amount deducted from the weight of the proteose precipitate. In every case, duplicate determinations were made to insure freedom from error. Deducting the combined weight of the proteoses, neutralization precipitate and antialbumid from the weight of the dry proteid used in the digestion, gives, with a fair degree of accuracy, the amount of peptone formed.

The only other point to be considered in this connection is the amount of proteoses introduced into each digestive mixture with the pepsin employed. This was determined in each case by a control experiment, in which a given weight of the pepsin was warmed at 38° C. with a suitable volume of dilute acid for the same length of time as in the digestion experiments. The fluid was then analyzed, the neutralization precipitate and proteoses being determined by the methods just described and the necessary deductions or corrections, although slight, made in each case.

EXPERIMENT I. The make-up of the several digestive mixtures in this experiment was as follows:

A. (Dialyzer). 400 c.c. 0·2 per cent. hydrochloric acid[1] containing 0·5 gram of a very strong scale pepsin, and 50 grams of moist coagulated egg-albumin, the latter containing 5·45 grams of dry proteid.

B. (Flask). 400 c.c. 0·2 per cent. hydrochloric acid with 0·5 gram scale pepsin and 50 grams moist albumin coagulum.

C. (Flask). 400 c.c. 0·2 per cent. hydrochloric acid with 0·5 gram scale pepsin and 25 grams moist albumin coagulum.

X. (Flask). 200 c.c. 0·2 per cent. hydrochloric acid and 1·5 grams of the scale pepsin, this mixture serving as a control by the analysis of which the necessary corrections could be made for the neutralization precipitate, proteoses, etc., introduced with pepsin into the several digestions.

[1] The hydrochloric acid used was made from chemically pure acid, and the sodium carbonate used later on for neutralization of the large volume of dilute acid surrounding the dialyzer, was also chemically pure.

The mixture (A) destined for the dialyzer was at first, like the others, warmed at 38° C. in a flask until the albumin was dissolved, when the fluid was transferred to a carefully tested parchment tube, the last portions being rinsed in with a little 0·2 per cent. acid. Complete solution of the coagulated albumin occurred in an hour's time, which may serve as an indication of the proteolytic strength of the artificial gastric juice. The parchment tube with its contents was suspended in the inner cylinder of the apparatus above described in direct contact with a little over 3 litres of 0·2 per cent. hydrochloric acid, the whole being kept at a temperature of 38° C. throughout the experiment, which lasted eleven hours. Further, from time to time small portions (300—400 c.c.) of the acid diffusate were withdrawn from the cylinder and fresh 0·2 per cent. acid added. At the expiration of the eleven hours, the contents of the parchment tube were carefully poured into a suitable vessel and the tube thoroughly rinsed, after which further ferment action was prevented by heating the mixture to boiling. The solution was then analyzed with the results shewn in the following table.

The 4 litres, or more, of acid diffusate, the contents of the cylinder plus the amounts withdrawn, were carefully neutralized with pure sodium carbonate, and then evaporated to a small volume. When quite concentrated, so much so that sodium chloride commenced to crystallize out, a precipitate made its appearance, evidently some proto or heteroalbumose which had diffused through the parchment membrane. The concentrated fluid was analyzed, the albumoses being determined by the method already described, with the results shewn in the accompanying table.

The digestions in the flasks *B*, *C* and *X* were kept at 38° C. for the same length of time as digestion *A*; *i.e.* eleven hours, after which further proteolytic action was stopped by heating the mixtures to boiling. Each was then analyzed with the results shewn in the table following, slight corrections being made from the data obtained in *X* for the albumoses, etc. introduced with the pepsin.

If the slow conversion of proteoses into peptone in artificial gastric digestion, previously commented on, is due wholly or in great part to an accumulation of the products of digestion, we certainly should observe a large increase in the amount of peptone formed in the dialyzer experiment. Any very noticeable increase in the above experiment, however, is wanting. There is, to be sure, as the results shew, 5·5 per cent. more peptone in *A* than in *B*, an increase which may doubtless be ascribed

to a partial withdrawal of the products of digestion by diffusion. But when we consider the length of time the digestion was continued (11 hours) and the large volume of acid diffusate, partially renewed from

EXPERIMENT I.

5·45 grams of dry albumin in *A* and *B*. 2·73 grams in *C*.

		Antialbumid		Neut. precipitate		Albumoses		Peptone	
		gram	p. c.	gram	p.c.	grams	p.c.	grams	p.c.
A	Dialyzer	0·1112	= 2·04	0·0249	= 0·45	2·9775	= 54·63		
A	Diffusate	0		0		0·2668	= 4·89		
A	Total	0·1112	= 2·04	0·0249	= 0·45	3·2443	= 59·52	2·0696	= 37·99
B	(Flask)	0·1118	= 2·05	0·0238	= 0·43	3·5490	= 65·11	1·7654	= 32·41
C	(Flask)	0·0521	= 1·90	0·0094	= 0·34	1·6948	= 59·79	1·0352	= 37·97

time to time, together with the necessary withdrawal from the parchment tube of more or less of the peptone formed, it is plain that in this experiment at least, peptonization has not been greatly accelerated by this closer approximation to the natural process. Between A and C there is a close similarity in results; which certainly shews the favorable effect of either direct dilution, or indirect dilution by withdrawal of the products of digestion as they are formed, on the proteolytic action of the ferment. But evidently more than this is needed to effect complete peptonization, or anything approaching this condition. It is to be further noticed, that so far as the formation of antialbumid and neutralization precipitate is concerned the withdrawal of the products of digestion has no appreciable effect. A point of considerable interest, however, brought out by this experiment, is the diffusibility of the albumoses. That proteoses are more or less diffusible has long been known. In the many articles published on the various forms of proteoses[1] this point has been commented on in a general way from time to time, but no quantitative experiments have been recorded. P. Horton Smith has likewise emphasized this diffusibility of proteoses[2] in a recent study of the albumoses and peptones formed in the peptonization of milk. In the above experiment (*A*), 59·52 per cent. of albumoses were found at the end of the eleven hours' digestion at 38° C. Of these, 54·63 per cent. were found in the contents of the parchment dialyzing tube, while 4·89 per cent. were present in the acid diffusate.

[1] See Kühne and Chittenden. *Zeitschrift für Biologie.*

[2] This *Journal.* Vol. XII. p. 54.

Or, expressing it in another way, of the amount of albumoses present at the end of the digestion 8·2 per cent. had diffused through the walls of the parchment tube in 10 hours at 38° C. Hence, from this it might perhaps be conjectured that the partial removal of the albumoses by their diffusibility, thus hurrying them from the presence of the ferment and consequently diminishing the material for the ferment to act upon, would tend to decrease the amount of true peptone formed. This diffusibility of the proteoses will be considered more in detail later on.

When we take into account the far greater diffusibility of true peptone, as compared with that of proteoses in general, it is plain from the above results that a comparatively large amount of peptone must have been withdrawn from the digestive fluid by dialysis, without, however, producing any very marked increase in the formation of peptone.

Experiment II. In this experiment, fluid egg-albumin was used, being prepared after the manner recommended by Schütz[1]. To every 300 c.c. of fresh egg-albumen, 4·2 c.c. hydrochloric acid of 1·12 specific gravity were added, the mixture shaken, and after standing some time freed from the precipitated globulin by filtration. The amount of albumin contained in the solution was determined by heating 10 c.c. of the fluid, suitably diluted, to boiling and then neutralizing exactly with a few drops of a dilute solution of sodium carbonate. The coagulum was collected on a filter, washed, dried, and weighed.

A. (Dialyzer). 350 c.c. 0·2 per cent. hydrochloric acid, with 0·5 gram of a strong scale pepsin, and 60 c.c. of the prepared fluid egg-albumin, equivalent to 5·655 grams of dry albumin.

B. (Flask). The same mixture as in A.

X. (Flask). A control experiment with pepsin-hydrochloric acid alone.

All three mixtures were warmed at 38° C. for ten hours; *A* in the dialyzer, or parchment tube, being surrounded by the same volume of 0·2 per cent. hydrochloric acid as in the preceding experiment (a little more than 3 litres) and under all the conditions described there, portions of the diffusate being withdrawn from time to time and fresh quantities of acid added. At the end of the ten hours, the contents of the flasks *B* and *X* were heated to boiling to destroy the ferment, the contents of the dialyzing tube were carefully removed and further action stopped by boiling, while the large volume of acid diffusate was neutralized and concentrated. The several solutions were then analyzed after the

[1] *Zeitschrift für physiol. chem.* Bd. IX. p. 581.

methods already described. The results are shown in the accompanying table, the necessary corrections having been made from the data obtained in *X*.

EXPERIMENT II.

5·655 grams of dry albumin in *A* and *B*.

		Antialbumid		Neut. precipitate		Albumoses		Peptone	
		gram	p.c.	gram	p.c.	grams	p.c.	grams	p.c.
A	Dialyzer	0·0165	= 0·29	0·0213	= 0·37	3·5248	= 62·33		
A	Diffusate	0		0		0·2295	= 4·05		
A	Total	0·0165	= 0·29	0·0213	= 0·37	3·7543	= 66·38	1·8629	= 32·95
B	(Flask)	0·0289	= 0·51	0·0150	= 0·26	3·7889	= 67·00	1·8222	= 32·22

Here, we see practically no difference in proteolytic action between the digestion in the flask and in the dialyzer; the results are almost identical. Further, in this digestion as in the preceding, we see that a certain amount of the albumoses have passed through the parchment walls of the dialyzer; 4·05 per cent. of the total proteid matter, or 6·1 per cent. of the albumoses present at the end of the digestion.

EXPERIMENT III. The proteid matter employed in this digestion was blood-fibrin, which had been thoroughly washed with water, boiled in water and extracted with cold and hot alcohol. Lastly, it was boiled again with water to displace the alcohol.

A. (Dialyzer). 400 c.c 0·2 per cent. hydrochloric acid, with 0·5 gram powdered pepsin and 25·0 grams of moist blood-fibrin, equivalent to 10·9 grams of dry proteid.

B. (Flask). Same mixture as in A.

X. (Flask). A control experiment with pepsin-acid alone.

EXPERIMENT III.

10·9 grams of dry fibrin in *A* and *B*.

		Antialbumid & neutral. precipitate		Proteoses		Peptone	
		gram	p.c.	grams	p.c.	grams	p.c.
A	Dialyzer	0·4352	= 4·0	5·5447	= 50·86		
A	Diffusate	0		0·3830	= 3·51		
A	Total	0·4352	= 4·0	5·9277	= 54·37	4·5371	= 41·62
B	(Flask)	0·4673	= 4·3	5·8729	= 53·88	4·5598	= 41·83

These digestions were warmed at 38° C. for 8 hours, in all other respects being treated in exactly the same manner as in the two preceding experiments. Antialbumid and the neutralization precipitate, however, were determined together.

Here, again, no difference is to be noticed in the amount of peptone formed in the two digestions, although there must have been a rapid removal of the diffusible products from the contents of the dialyzer tube, as is evidenced by the diffusion of 6·4 per cent. of the formed proteoses.

EXPERIMENT IV. In this experiment, the pepsin solution employed was prepared by warming scrapings from the mucous membrane of a pig's stomach with 0·4 per cent. hydrochloric acid, for some time, at 38° C., purifying the solution by dialysis, and finally bringing the acidity up to 0·2 per cent. HCl. The proteid matter used was blood-fibrin prepared as in the preceding experiment.

A. (Dialyzer). 200 c.c. of the prepared pepsin-acid solution and 200 c.c. 0·4 per cent. hydrochloric acid, making a total of 400 c.c. 0·3 per cent. hydrochloric acid with the contained pepsin. To this were added 25 grams of the prepared blood-fibrin, equivalent to 8·2 grams of dry proteid.

B. (Flask). Same composition as A.

X. (Flask). 200 c.c. of the prepared pepsin-acid solution and 200 c.c. 0·4 per cent. hydrochloric acid.

These mixtures were warmed at 38° C. for 9 hours. Solution of the fibrin was very rapid in both *A* and *B*, indicating that the pepsin was quite active, but as seen from the results, the formation of peptone was not large. Neither was there any noticeable difference in the relative proportion of proteoses and peptone in *A* and *B*.

EXPERIMENT IV.

8·2 grams of dry fibrin in *A* and *B*.

		Antialbumid & neutral. precipitate		Proteoses		Peptone	
		gram	p. c.	grams	p. c.	grams	p. c.
A	Dialyzer	0·7901	= 9·6	5·2203	= 63·60		
	Diffusate	0		0·1768	= 2·15		
	Total	0·7901	= 9·6	5·3971	= 65·75	2·0128	= 24·5
B	(Flask)	0·9020	= 11·0	5·2494	= 63·00	2·0486	= 24·9

Experiment V. The pepsin-hydrochloric acid solution was the same as that used in the preceding experiment. The proteid was coagulated egg-albumin.

A. (Dialyzer). 200 c.c. of the prepared pepsin-acid solution, 200 c.c. 0·4 per cent. hydrochloric acid and 50 grams of the moist albumin coagulum, containing 5·5 grams of dry proteid.

B. (Flask). Same composition as A.

X. (Flask). Pepsin-acid control.

The mixtures were warmed at 38° C. for 8 hours and then analyzed with the following results:

Experiment V.

5·50 grams of dry albumin in *A* and *B*.

		Antialbumid and neutral. precipitate		Albumoses		Peptone	
		gram	p. c.	grams	p. c.	grams	p. c.
A	Dialyzer	0·3753	= 6·8	3·9882	= 72·5		
	Diffusate	0		0·2834	= 5·1		
	Total	0·3753	= 6·8	4·2716	= 77·6	0·8531	= 15·5
B	(Flask)	0·2913	= 5·3	3·9034	= 71·0	1·3053	= 23·7

Here, for some reason, there appears to have been less proteolytic action in *A* than in *B*; seen not only in the lesser yield of peptone, but also in the larger percentage of antialbumid and neutralization precipitate. It is difficult to find a rational explanation of this peculiar result, unless it is dependent upon the rapid diffusion of a portion of the albumoses thus diminishing the amount of primary cleavage products to be converted into peptone, or possibly to a dilution the of contents of the dialyzer tube attendant upon the rapid exit of the diffusible products formed. Against this latter explanation, which is theoretically quite plausible, we have the lack of any similar observation in the several other experiments recorded.

The results of the five experiments described, so far as they bear on the relative formation of proteoses and peptone, may be conveniently exhibited in the following table:

Experiment	Proteoses		Peptone	
	Dialyzer and Diffusate	Flask	Dialyzer and Diffusate	Flask
No. I.	59·52 %	65·11 %	37·99 %	32·41 %
„ II.	66·38	67·00	32·95	32·22
„ III.	54·37	53·88	41·62	41·83
„ IV.	65·75	63·00	24·50	24·90
„ V.	77·60	71·00	15·50	23·70

It is thus evident that the slow and incomplete peptonization, so often commented upon as characteristic of artificial gastric digestion, is not materially modified by this closer approach to the natural process. Under the conditions described, the several digestions carried on in the dialyzer tubes were certainly accompanied by a fairly rapid withdrawal of the diffusible products of digestion, yet no appreciable difference is to be noted in the proportion of the final products. The parchment dialyzer tubes were of small diameter and quite long, consequently when suspended in the 3—4 litres of warm 0·2 per cent. acid the conditions for rapid diffusion were very favorable. Yet, under such circumstances, the partial removal of the products of digestion thus accomplished appears to have influenced but little the proteolytic action of the ferment. In other words, the results here obtained favor the view that the conversion of the primary products of gastric digestion into true peptone is a slow and gradual process, even under the most favorable circumstances. It is our belief that complete peptonization is not a property of gastric digestion, either in the artificial or in the natural process. The action of pepsin-hydrochloric acid is rather a preliminary stage in proteolytic digestion; a preparation for the more important changes peculiar to the small intestine, in which the more energetic alkaline trypsin solution plays a conspicuous part. In favor of this view we have the well-known fact that some natural proteids are exceedingly resistent to the ordinary solvent action of pepsin-hydrochloric acid, while readily attacked by pancreatic juice. This is true to a certain extent of the proteids of muscle tissue. Cooked beef, for example, is far less readily dissolved by artificial gastric juice than by pancreatic juice, while blood-fibrin, on the other hand, succumbs as readily to pepsin-hydrochloric acid as to trypsin solutions, although the formation of peptone in the former case is far less rapid. The latter constitutes one of the striking points of difference between the gastric and pancreatic

digestion of proteids. In gastric digestion, a digestible proteid may be quickly transformed into primary proteoses and then more slowly into the secondary proteose, after which conversion into peptone progresses very slowly. With pancreatic digestion, on the other hand, a digestible proteid may be more or less rapidly converted into soluble proteoses, these in turn being quickly and more or less completely transformed into true peptone. Again, it seems quite possible that the proteoses formed in gastric digestion may be directly absorbed to a certain extent. They are certainly more closely related to the proteids found in the circulating blood than are true peptones, and although less diffusible through vegetable parchment than the latter, they may perhaps be absorbed from the alimentary tract in virtue of the selective activity of the epithelial cells, especially in the intestine. If so, it is easy to see that the changes incidental to a reconversion into the circulating proteids of the blood would be less profound than in the absorption of true peptone.

While, then, our results favor the view that the lack of complete peptonization in artificial gastric digestion is not due to accumulation of the products of digestion, but is rather an inherent quality of pepsin-hydrochloric acid under all circumstances, it is not to be considered that our dialyzer digestions approach the conditions existent in the alimentary tract other than in the crudest way. In natural digestion, the products formed are unquestionably removed from the digestive tract with far greater rapidity and much more completely than is possible in our artificial digestions. Further, in the natural process there is a more or less continual addition of fresh quantities of gastric juice and, doubtless, in a concentrated form. These conditions cannot well be imitated in an artificial digestion, and what their influence on peptonization in the stomach may be, can only be conjectured. This much, however, certainly seems plausible; viz. if complete peptonization is characteristic of gastric digestion and our failure to observe it in ordinary artificial digestions is due to accumulation of the products of digestion, then certainly some results favorable to this view should have been obtained in our dialyzer experiments. On the other hand, it is not to be necessarily inferred that withdrawal of the products of digestion, as by dialysis, is without influence on the action of the ferment. Doubtless, with more concentrated solutions, the results of the dialyzer experiments would have shewn some favorable influence exerted by the partial withdrawal of the products of digestion; but our aim has been not to study the influence of dialysis on gastric digestion

but simply to ascertain whether the lack of complete peptonization in gastric digestion is due, to any great extent, to the accumulation of the products of digestion. And with this end in view our experiments have been conducted under those conditions which experience has taught us lead to the largest yield of peptone; viz. a fairly large proportion of dilute acid (0·2 per cent. HCl), which necessarily means a fair degree of dilution. Under such conditions, withdrawal of a portion of the products of digestion is apparently without influence on peptonization by pepsin-hydrochloric acid, or at least does not lead to complete peptonization.

2. *The relative proportion of proteoses and peptone in natural gastric digestion in the human stomach.*

In any attempt to study the formation of proteoses and peptone in gastric digestion, as it takes place in the human stomach, we are at once confronted with two difficulties. The first and more important one is the possible rapid removal of the more diffusible products of digestion, *i.e.*, the peptone, from the stomach contents, while the less diffusible proteoses naturally remain, and on analysis give misleading results as to the relative formation of proteoses and peptone. On this question of removal of diffusible products from the stomach by absorption, there is more or less diversity of opinion. Lea[1], for example, assumes that "normally the products of digestion, whether proteid or carbohydrate, are never met with in either the stomach or intestine in other than the smallest amounts, frequently to be described as merely traces." Other writers claim a far less rapid withdrawal of the products of digestion from the stomach than from the intestine. Indeed, as is well known, the facilities for absorption from the intestine, as indicated by structural peculiarities, are far better than are to be found in the stomach. No one, of course, doubts the absorption of diffusible products from the stomach to a greater or less extent, but it may well be questioned whether such absorption is as complete as indicated by Lea's statement. If it is, then certainly any analysis of the stomach contents would simply shew a preponderance of the non-diffusible products of digestion and hence fail to throw any light on the relative formation of proteoses and peptone in natural gastric digestion. The second difficulty to be considered, is the natural onward passage of the stomach contents in to the duodenum. This tendency obviously limits

[1] This *Journal*. Vol. XI. p. 240.

the length of time a test meal can be allowed to remain in the stomach, since naturally the more fluid portions of the acid chyme with its semi-digested proteids would pass from the stomach first, and thus be lost for analysis. With a full recognition of these possible drawbacks, a few experiments[1] have been tried in order to gain some insight on peptonization in the living stomach.

The first experiment was simply a qualitative one, with a view to obtaining a general idea of the results to be expected. The subject was a young man in perfect health, with vigorous digestive powers. Three hours after a light breakfast, the stomach was washed out with a litre or more of warm water, until the washings came away perfectly clear and free from acidity. In washing out the stomach and in the withdrawal of the stomach contents at the end of the digestive period, a rubber tube used as a syphon was employed with a funnel attached, after the manner recommended by Ewald. After the stomach was cleansed, 88 grams of moist coagulated egg-albumin, thoroughly broken up by trituration in a mortar, were taken into the stomach, a sprinkle of salt being added to increase its palatability. This amount of moist coagulum contained about 10 grams of dry albumin.

At the end of an hour's digestion, the contents of the stomach were withdrawn, about half a litre of water being used to accomplish this and to rinse the stomach. The fluid was tinged slightly yellow with bile and contained considerable mucus, but no particles of undissolved albumin were to be seen. The fluid was quickly boiled to check further proteolytic action and then filtered. It was next neutralized with sodium carbonate and concentrated to a small volume. Only a small neutralization precipitate was obtained. The concentrated fluid, containing any albumoses and peptone present, was saturated while hot with ammonium sulphate, and yielded a gummy precipitate sticking to the rod and sides of the dish. In the filtrate, a distinct and fairly strong biuret reaction was obtained, shewing the presence of true peptone. The albumose precipitate was fairly large in amount and on being tested was found to contain considerable protoalbumose, as well as deuteroalbumose. Hence, from this experiment, it is evident that peptone is not entirely removed from the stomach contents as soon as it is formed, and further, that complete solution of the proteid matter is not by any means synonymous with complete peptonization in natural

[1] These experiments were conducted by Dr George S. Woodward on himself, the analysis of the stomach contents having been made mainly by him, under the writer's supervision.

gastric digestion any more than in the artificial process. Judging from the intensity of the biuret reaction, the amount of peptone present in the solution must have been considerably less than the amount of albumose, although of course we have no clue as to the amount of peptone that may have been absorbed, and thus removed from the stomach contents.

Other preliminary trials shewed us that by using such finely divided albumin, solution took place very quickly, and that in an hour's time either the particles were entirely dissolved or else had escaped into the duodenum. It will be remembered, however, that in some of our artificial digestions in flasks, the coagulated albumin was entirely dissolved in an hour at 38° C. If, however, the albumin was introduced into the stomach in larger lumps, thus retarding somewhat the rate of proteolytic action, some of the undissolved particles of albumin invariably clogged the stomach tube and thus caused much trouble in the removal of the stomach contents.

First Quantitative Experiment. 138 grams of finely coagulated egg-albumin, equal to 16 grams of dry albumin, were ingested at 11.30 a.m., the stomach having been thoroughly washed out a short time before. About three-fourths of an hour thereafter, the contents of the stomach were removed by lavage and the mixture quickly brought to boiling, and filtered from more or less mucus which was present. The clear filtrate was then neutralized, yielding a little acid-albumin, and concentrated to a small volume. The albumoses present in the solution were then separated by saturation with ammonium sulphate and the amount determined, by the method already described. As a result, 1·413 grams of albumoses were found in the stomach contents.

The ammonium sulphate-saturated filtrate and washings from the albumose precipitate, containing the peptone present, were diluted somewhat with water and then treated with a saturated solution of barium hydroxide until the ammonium sulphate was entirely decomposed. At last, a slight excess of barium hydroxide was added and the mixture heated gently for some time, until the free ammonia was entirely removed from the fluid. The solution was then filtered from the heavy precipitate of barium sulphate, the latter washed thoroughly both by decantation with large volumes of water and on the filter with warm water to completely remove all traces of peptone. The filtrate and washings were then concentrated to a small volume, the fluid being made exactly neutral by the addition of a drop or two of very dilute sulphuric acid. The barium sulphate was next removed by filtration, after which

the filtrate and washings were evaporated to dryness in a weighed capsule, and dried at 110° C. until of constant weight. The residue, so obtained, containing all of the peptone together with sodium chloride and other inorganic salts, with perhaps some organic matter, weighed 1·835 grams. In this residue, peptone was next determined by use of the phosphotungstic acid method[1]. It was dissolved in a little warm water, the solution acidified with sulphuric acid so that the mixture contained approximately 6 per cent. H_2So_4 and precipitated with a large excess of phosphotungstic acid. After standing over night, the peptone compound was filtered off, washed with 5 per cent. sulphuric acid until the excess of phosphotungstic acid was entirely removed, then decomposed with warm baryta water. The resultant solution, containing the peptone-baryta compound with some little excess of baryta, was made as near neutral as possible with dilute sulphuric acid, the solution concentrated, and again tested to make sure of neutrality. The clear solution, freed from all barium sulphate by filtration, was concentrated to a very small bulk and precipitated by a large volume of absolute alcohol. This final precipitate of peptone was then dissolved in a little water and the solution evaporated to dryness in a weighed capsule, after which it was dried at 110° C. until of constant weight.

The peptone thus obtained weighed 0·8365 gram. Unquestionably it still contained some inorganic salts, for peptone never yet has been separated, by this or any other method, without containing some admixture of mineral matter. This, however, may not be more than enough to compensate for the natural loss of peptone to be expected in such a long and tedious method of separation.

Considering, now, the results as obtained, we see that the stomach contents contained, at the time they were withdrawn from the stomach, 1·4130 grams of albumoses and 0·8365 gram of peptone, the relative proportion being expressed by 62 per cent. of albumoses and 37 per cent. of peptone, calculated on the 2·2495 grams of soluble products recovered. The amount of dry albumin introduced into the stomach was 16 grams, so that only about one-seventh was recovered as albumoses and peptone. There should be added to this the weight of the neutralization precipitate, which, however, was slight; but at the time the experiment was tried the sole point in mind was to obtain the relative amounts of albumoses and peptone. How much of this deficit is due to absorption of the more diffusible products, and how much to passage

[1] Kühne and Chittenden. "Ueber die Peptone." *Zeitschrift für Biologie.* Bd. XXII. p. 440.

into the intestine, can only be conjectured. A certain amount of the deficit must also be ascribed to the lack of complete withdrawal of products from the stomach. The main point sought for, however, in these experiments, was the rate of peptonization. Digestion is here obviously more rapid than in a flask, or in a dialyzer; this would naturally be expected, but we see much the same relative proportion of albumoses and peptone in the soluble products found as in the artificial digestions. The results certainly do not point to complete and rapid peptonization as a necessary feature of gastric digestion in the stomach.

Second Quantitative Experiment. 165 grams of finely divided, coagulated egg-albumin were introduced into the stomach at 11.30 a.m., the stomach having been previously washed out. This amount of coagulum was equivalent to about 19 grams of dry albumin. At 12.30 p.m., one hour after, the contents of the stomach were syphoned off and the ferment killed by boiling. In this case, some little undissolved albumin was noticed, and on neutralization of the acid fluid, quite a heavy flocculent precipitate of acid-albumin resulted, shewing that digestion had not gone so far as in the preceding experiment, although the latter was of shorter duration. Further, on concentration of the neutralized fluid, a little coagulum separated, doubtless coming from the coagulation of some dissolved acid-albumin or heteroalbumose. Evaporation of the filtrate from this coagulum gave on drying at 110° C. a residue weighing 3·937 grams. From this was separated, by the methods already described, 2·293 grams of albumoses. Assuming that the difference between the weight of albumoses found and the weight of the total residue was all peptone, it would give for the latter only 1·644 grams. But in reality, the actual amount of peptone present was 0·698 gram. This would shew 76 per cent. of albumoses against 23 per cent. of peptone in the soluble products recovered. Plainly then, as before stated, peptone is not absorbed from the stomach as soon as formed, and can be detected in appreciable quantity in the stomach contents. Again, in the two quantitative experiments reported, the albumoses are found considerably in excess of the peptone, as in the artificial digestion experiments, although we can make no definite statements as to the amount of peptone that may have been absorbed. In our opinion, however, the results, so far as they extend, lend favor to the view that complete peptonization is not necessarily a feature of natural gastric digestion, any more than of the artificial process; although it is undoubtedly true, that the successive changes characteristic of gastric digestion are more quickly accomplished in the natural process. Further,

this view would well accord with the position which gastric digestion may be assumed to occupy in reference to the proteolytic changes going on in the alimentary tract, viz.: as a preliminary step preparatory to the more profound changes characteristic of pancreatic digestion. And just here it may be mentioned incidentally, that the gradual diffusibility of the albumoses, brought out by our experiments, may serve as a means for their partial utilization by absorption, without necessarily involving a complete conversion into the more diffusible peptone.

3. *The diffusibility of proteoses and peptone.*

In the original article describing the individual albumoses[1], attention was called to the fact that these bodies, like peptone, are diffusible, although not readily so. Still, it was noticed even then, that by long-continued dialysis of albumose preparations the loss amounted to considerable. This peculiarity of albumoses, or of proteoses in general, does not appear to have been widely recognized. In fact, in many published accounts of the albumoses, it is not uncommon to meet with the statement that these bodies resemble albumin by reason of the fact that they will not diffuse through membranes, and hence differ very markedly from the readily diffusible peptone. As a matter of fact, proteoses are diffusible, and in this respect may be considered rather as occupying a position between the non-diffusible albumin and the readily diffusible peptone. Still, quantitative experiments demonstrating the extent of this diffusibility have been wanting[2], and consequently it has been the object of the following experiments to obtain a few quantitative data regarding the diffusibility of proteoses and peptone formed by proteolytic action.

In the digestion experiments carried on in parchment dialyzer tubes, already recorded, it was found that the proteoses resulting from the digestion of egg-albumin and blood-fibrin in pepsin-hydrochloric acid, shewed a diffusibility under the given conditions amounting to 3·2—8·2 per cent. of the total proteoses formed. Eliminating the single low result (3·27 per cent.) the average of the other four closely agreeing results shews a diffusibility amounting to 6·8 per cent. for 9 hours dialysis at 38° C. Here it is to be remembered that diffusion of the

[1] Kühne and Chittenden. "Ueber Albumosen." *Zeitschrift für Biologie.* Bd. xx. p. 27.

[2] Since this was written, a paper by Kühne has appeared, "Erfahrungen über albumosen und Peptone." *Zeitschrift für Biologie.* Bd. xxix. p. 1, in which are recorded some experiments on the diffusibility of albumoses.

several albumoses commenced at the very moment of their formation and in the presence of 0·2 per cent. hydrochloric acid; a fact which may have some influence upon the rate of diffusion. Again, it may be that these bodies are influenced in their diffusibility by the presence or diffusion of other products, notably peptone, formed at the same time.

In the experiments about to be described, parchment paper tubes[1] were employed, having a diameter of 1·2 inches, the same as those used in the dialyzer experiments already recorded. It is to be remembered, however, that while these tubes are all apparently alike, they may differ from each other somewhat in the thickness of their walls, and doubtless somewhat in their permeability. On this account, if for no other reason, minor differences at least may be expected in the results. Naturally, every tube used was carefully tested prior to the experiment, to make sure that it was entirely free from leakage.

The method employed was very simple; a given weight of the pure substance to be tested, previously dried at 110° C., was dissolved in a definite volume of water, usually in such proportion as to make approximately a 1 per cent. solution. It was then introduced into the parchment tube, the last portions being carefully rinsed in with a little water, and the tube suspended in a large cylinder (of either 4½ litres capacity or 17 litres), filled with water kept at a constant temperature, and with a slow current of fresh water at the same temperature continually passing through it.

At the end of the dialysis, the amount of substance which had diffused was determined by evaporating the contents of the parchment tube, together with all of the washings required in thoroughly cleansing it, to a small volume, when it was transferred to a weighed capsule, evaporated to dryness, and finally dried in an air-bath at 110° C. until of constant weight. The difference between the weight of the substance originally taken and the amount recovered gives with a fair degree of accuracy the rate of diffusion.

The albumoses and peptone used in the following experiments were prepared from coagulated egg-albumin by digestion with pepsin-hydrochloric acid, and were separated by the usual methods, already well known. They were as pure as such preparations can well be obtained, none of them having more than 2 per cent. of ash, and this presumably composed wholly of non-diffusible salts, since all of the bodies had been purified by long-continued dialysis.

[1] Obtained from the manufacturer, C. Brandegger, of Ellwangen, Würtemberg.

EXPERIMENT I. *With protoalbumose.*

	A	*B*
Duration of dialysis	8 hours	8 hours
Temperature	38° C.	10° C.
Water surrounding dialyzer tube	4·5 litres	4·5 litres
Substance used (in 200 c.c. H_2O)	1·9876 grams	1·9327 grams
,, recovered	1·8863 ,,	1·8829 ,,
,, diffused	0·1013 ,,	0·0498 ,,
,, ,,	5·09 per cent.	2·57 per cent.

EXPERIMENT II. *Mixture of proto and deuteroalbumose*[1].

	A	*B*
Duration of dialysis	6 hours	6 hours
Temperature	38° C.	7° C.
Water surrounding dialyzer tube	4 litres	4 litres
Substance used (in 200 c.c. H_2O)	2·4504 grams	2·0131 grams
,, recovered	2·2741 ,,	1·9623 ,,
,, diffused	0·1763 ,,	0·0508 ,,
,, ,,	7·2 per cent.	2·5 per cent.

EXPERIMENT III. *Mixture of proto and deuteroalbumose.*

	A	*B*
Duration of dialysis	8 hours	8 hours
Temperature	38° C.	8° C.
Water surrounding dialyzer tube	4·5 litres	4·5 litres
Substance used (in 200 c.c. H_2O)	1·8419 grams	2·2263 grams
,, recovered	1·7332 ,,	2·1849 ,,
,, diffused	0·1087 ,,	0·0414 ,,
,, ,,	5·9 per cent.	1·85 per cent.

EXPERIMENT IV. *Mixture of proto and deuteroalbumose.*

	A	*B*
Duration of dialysis	6 hours	7 hours
Temperature	38° C.	38° C.
Water surrounding dialyzer tube	17 litres	17 litres
Substance used (in 200 c.c. H_2O)	2·3275 grams	3·6163 grams
,, recovered	2·1561 ,,	3·3456 ,,
,, diffused	0·1714 ,,	0·2707 ,,
,, ,,	7·36 per cent.	7·48 per cent.

[1] This mixed albumose was the acetic acid precipitate, obtained on adding a little salt-saturated acetic acid to a solution of albumoses, from which heteroalbumose and the greater portion of the protoalbumose had been previously removed by saturation of the neutral fluid with sodium chloride. This precipitate, which is well known to be a mixture of proto and deuteroalbumose with the former predominating, was purified by dissolving in water, neutralizing, and dialyzing in running water until the diffusible salts were entirely removed. The solution was then concentrated and the albumoses precipitated by alcohol, washed with alcohol, ether, and dried at 110° C.

EXPERIMENT V. *Deuteroalbumose.*

	A	*B*
Duration of dialysis	7 hours	7 hours
Temperature	38° C.	10° C.
Water surrounding dialyzer tube	4·5 litres	4·5 litres
Substance used (in 200 c.c. H_2O)	2·5121 grams	2·5162 grams
,, recovered	2·4565 ,,	2·4629 ,,
,, diffused	0·0556 ,,	0·0533 ,,
,, ,,	2·21 per cent.	2·11 per cent.

EXPERIMENT VI. *Deuteroalbumose.*

Duration of dialysis	3 hours
Temperature	38° C.
Water surrounding dialyzer tube	17 litres
Substance used (in 150 c.c. H_2O)	1·2977 grams
,, recovered	1·2737 ,,
,, diffused	0·0240 ,,
,, ,,	1·84 per cent.

EXPERIMENT VII.

a. With protoalbumose.

b. With a mixture of proto and deuteroalbumose, *i.e.* the above described acetic acid precipitate.

In this experiment, the dialysis was carried on in bags of parchment paper, suspended in the water. The parchment was considerably thicker and heavier than that composing the parchment tubes ordinarily used.

	A Protoalbumose	*B* Mixed albumoses
Duration of dialysis	8 hours	7 hours
Temperature	38° C.	38° C.
Water surrounding dialyzer tube	17 litres	17 litres
Substance used (in 200 c.c. H_2O)	1·7373 grams	4·2370 grams
,, recovered	1·5986 ,,	4·0603 ,,
,, diffused	0·1387 ,,	0·1767 ,,
,, ,,	7·9 per cent.	4·17 per cent.

EXPERIMENT VIII. *With peptone.*

	A	*B*[1]
Duration of dialysis	6 hours	6 hours
Temperature	38° C.	38° C.
Water surrounding dialyzer tube	17 litres	17 litres
Substance used (in 150 c.c. H_2O)	1·5205 grams	1·5530 grams
,, recovered	1·3526 ,,	1·3842 ,,
,, diffused	0·1679 ,,	0·1688 ,,
,, ,,	11·0 per cent.	10·80 per cent.

[1] A different sample of peptone, prepared, however, in essentially the same manner as *A*.

From these few data we see more or less of a confirmation of the results obtained in the dialyzer-digestion experiments; indeed, the average rate of diffusion in the above seven experiments with albumoses is much the same as noticed in the preceding digestion experiments. We may conclude, then, that albumoses, either separately or collectively, are capable of passing through parchment paper with a fair degree of rapidity, *i.e.*, they are diffusible, and their diffusibility is controlled or modified by the character of the conditions under which the experiments are tried. Thus, elevation of temperature alone causes a marked increase in the rate of diffusion, the ratio being in the neighbourhood of 3 to 1 for the temperatures 38° C. and 8° C. Very remarkable, however, is the behavior of deuteroalbumose. According to all preconceived ideas this body should have a much higher endosmotic equivalent than a primary albumose, yet our results shew that pure deuteroalbumose, under the conditions of our experiments, diffuses less rapidly than protoalbumose. As we fail to find any error in the work, we are forced to accept the correctness of the results, although there is some evidence that a mixture of proto and deuteroalbumose diffuses more rapidly than protoalbumose alone. True peptone, as seen from the above experiments, is, as would be expected, more diffusible than either of the two antecedent bodies.

Obviously, we cannot draw any definite conclusions from these results obtained with a parchment membrane, as to the absorption of albumoses in the alimentary tract. As Waymouth Reid[1] has well pointed out, we have to deal in a living membrane with an absorptive force, doubtless dependent upon protoplasmic activity and comparable, in part at least, to the excretive force of a gland cell. Moreover, the wonderful power of selection residing in the protoplasm of the epithelial cells may be even more potent than we can imagine.

Omitting, then, all attempts to draw any broad deductions from the few results recorded above, we may simply state that the albumoses formed in gastric digestion, like true peptone, possess a certain power of osmosis through vegetable parchment, although to a lesser degree. But even here, our results are to be taken, not as an exact quantitative expression of osmotic power, but rather as a general indication of the rate of diffusibility under certain definite conditions. For not only will temperature exercise a potent influence in determining the extent of diffusion, but the strength of the albumose solution, the volume of the surrounding water, the surface exposure, the thoroughness with which

[1] This *Journal*. Vol. xi. p. 312.

the water in the diffusate is changed, and even the character of the parchment paper itself, all exert, to a certain extent, a modifying influence upon the rate of diffusion. Thus we have found noticeable quantitative variations in using different grades and varieties of parchment paper, but we have reported more fully the results obtained with the parchment paper tubes, because they are more widely used for experiments of this character and the results have therefore a more practical value.

Diffusibility of gelatoses.

In this connection, we append here a short series of results obtained in a study of the diffusibility of gelatoses, prepared by the digestion of gelatin with artificial gastric and pancreatic juice. The preparations employed were those previously analyzed and described in a former paper "on the primary cleavage products[1] formed in the digestion of gelatin." These results may be of interest, not only as extending our knowledge of the diffusibility of the primary products resulting from the digestion of proteids and albuminoids, but also from the fact that gelatoses must frequently be formed by the action of bacteria, when gelatin is used as the basis of a culture medium, and it becomes a point of some practical importance to know regarding their diffusibility.

The methods employed were identical with those already described; the dialyzers being made from the parchment paper tubes of Brandegger, and the gelatoses dissolved so as to make approximately a 1 per cent. solution.

A. *Protogelatose, formed in gastric digestion.*

EXPERIMENT I.

	A	*B*
Duration of dialysis	8 hours	8 hours
Temperature	38° C.	12° C.
Water surrounding dialyzer tube	4·5 litres	4·5 litres
Substance used (in 150 c.c. H_2O)	1·5508 grams	1·5228 grams
„ recovered	1·5119 „	1·4860 „
„ diffused	0·0389 „	0·0368 „
„ „	2·50 per cent.	2·41 per cent.

[1] Chittenden and Solley. This *Journal.* Vol. XII. p. 23.

Experiment II.

	A	*B*
Duration of dialysis	10 hours	10 hours
Temperature	38° C.	12° C.
Water surrounding dialyzer tube	4·5 litres	4·5 litres
Substance used (in 200 c.c. H_2O)	2·2149 grams	2·2607 grams
,, recovered	2·1374 ,,	2·2065 ,,
,, diffused	0·0775 ,,	0·0542 ,,
,, ,,	3·49 per cent.	2·39 per cent.

B. *Protogelatose, formed in pancreatic digestion.*

Experiment III.

	A	*B*
Duration of dialysis	8 hours	8 hours
Temperature	38° C.	12° C.
Water surrounding dialyzer tube	4·5 litres	4·5 litres
Substance used (in 200 c.c. H_2O)	2·2055 grams	2·4433 grams
,, recovered	2·1023 ,,	2·3644 ,,
,, diffused	0·1032 ,,	0·0789 ,,
,, ,,	4·67 per cent.	3·22 per cent.

Experiment IV.

	A	*B*
Duration of dialysis	4 hours	8 hours
Temperature	38° C.	38° C.
Water surrounding dialyzer tube	17 litres	17 litres
Substance used (in 200 c.c. H_2O)	2·1231 grams	3·2785 grams
,, recovered	2·0646 ,,	3·1701 ,,
,, diffused	0·0585 ,,	0·1084 ,,
,, ,,	2·75 per cent.	3·30 per cent.

From these results it would appear that while protogelatose is fairly diffusible, it has a somewhat lower endosmotic equivalent than the corresponding albumose. It is further apparent that the gelatose is not so greatly influenced in its diffusibility by the rise of temperature as is protoalbumose, although in two out of three cases the difference in the results obtained at 12° C. and 38° C. is distinctly marked.

Doubtless, a wider study of the diffusibility of proteoses, and of the hydration products resulting from the proteolysis of the albuminoids, would shew that all of these bodies are more or less diffusible, although differing, perhaps, in their individual endosmotic equivalents.

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